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Anandamide inhibits excitatory transmission to rat substantia gelatinosa neurones in a manner different from that of capsaicin

Ceng Luo[a], Eiichi Kumamoto[a,*], Hidemasa Furue[a], Jun Chen[b], Megumu Yoshimura[a]

[a]Department of Physiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan
[b]Department of Anatomy and K.K. Leung Brain Research Centre, Fourth Military Medical University, Xi'an 710032, China

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Abstract

Actions of anandamide (10 μM) were examined on monosynaptic glutamatergic transmission from the periphery to substantia gelatinosa (SG) neurones in adult rat spinal cord slices using the whole-cell patch-clamp technique. In 64% of neurones examined, Aδ-fibre-evoked excitatory postsynaptic currents (EPSCs) were depressed (in amplitude; by 32 ± 4%, $n = 21$) by anandamide. On the contrary, an inhibitory action on C-fibre-evoked EPSCs was observed in only 31% of neurones tested; this magnitude (17 ± 3%, $n = 4$) was less than that of Aδ-fibre EPSCs ($P < 0.05$). A cannabinoid-receptor agonist, WIN 55,212-2 (5 μM), exhibited similar actions on the EPSCs. In a neurone with minimal effects of anandamide on C-fibre EPSCs, capsaicin (1 μM) largely depressed the EPSCs ($n = 3$); Aδ-fibre EPSCs were little affected by capsaicin. It is concluded that unlike capsaicin, anandamide inhibits more effectively Aδ-fibre than C-fibre-mediated excitatory transmission in the SG, possibly through the activation of the cannabinoid receptor.

Keywords: Pain; Anandamide; Capsaicin; Glutamatergic transmission; Primary afferent; Slice; Dorsal horn; Patch-clamp

When administered intrathecally, cannabinoids produce antinociceptive effects in acute pain models through the activation of the cannabinoid receptor (see [14] for review). For instance, applying an agonist, WIN 55,212-2 (WIN-2), of the receptor resulted in antinociception in the tail-flick test in adult rats [7]. This is possibly due to the activation of a subtype, CB1, of the cannabinoid receptor in the spinal dorsal horn, because expression of this receptor has been demonstrated there by immunohistochemistry [4]. The superficial dorsal horn, especially substantia gelatinosa (SG, lamina II of Rexed) of the spinal cord, plays an important role in the modulation of nociceptive transmission through fine myelinated Aδ and unmyelinated C primary afferents from the periphery (see [19] for review). The cellular mechanism for the antinociception of cannabinoids is thought to be an inhibition of the glutamatergic transmission to SG neurones, because this is the case of action at the spinal level of analgesics such as baclofen [2], 5-HT [8] and nociceptin [10]. In addition, cannabinoids have been shown to inhibit glutamatergic transmission in the central nervous system including the periaqueductal grey (PAG) [18] (see [3] for review). In support of this idea, very recently, Morisset and Urban [12] have demonstrated in juvenile rats that cannabinoids inhibit glutamatergic transmission to SG neurones through primary afferents. Although it is possible that like baclofen [2] and nociceptin [10], cannabinoids inhibit each of the Aδ- and C-fibre transmissions to a different extent, this was not examined in their studies. It is important to address this issue, because following ovariectomy-induced hyperalgesia, there was a remarkable difference in the expression of a specific type of 5-HT receptors between Aδ and C primary-afferent terminals innervating SG neurones, indicating a distinction in the modulation of pain transmission between the two types of fibre terminals [8].

We have previously demonstrated in rat SG neurones that capsaicin markedly enhances the frequency of miniature excitatory postsynaptic currents (mEPSCs) [20] and depresses the amplitude of C-fibre- but not Aδ-fibre-evoked excitatory postsynaptic currents (eEPSC) [21]. Although capsaicin does not exist endogenously, Zygmunt et al.

* Corresponding author. Tel.: +81-952-34-2273; fax: +81-952-34-2013.
E-mail address: kumamoto@post.saga-med.ac.jp (E. Kumamoto).

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[22] have suggested that an endocannabinoid, *N*-arachidonoylethanolamide (anandamide), may be an endogenous ligand of the capsaicin receptor in vascular preparations. If this is the case in the SG, anandamide is expected to have a similar effect to that of capsaicin. The aim of the present study was to know the action of anandamide on each of Aδ- and C-fibre eEPSCs in SG neurones and to compare this result with that of capsaicin.

All the experimental procedures involving rats were approved by the institutional Animal Use and Care Committee. The method used for obtaining an adult rat spinal cord slice that retained an attached dorsal root has been described elsewhere [9,10]. The Krebs solution contained: 117 mM NaCl; 3.6 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgCl_2$; 1.2 mM NaH_2PO_4; 25 mM $NaHCO_3$; and 11 mM glucose. SG neurones were identified by their location, as reported previously [9,10]. Blind whole-cell patch-clamp recordings were made from the SG neurones with patch-pipette electrodes having a resistance of 8–12 MΩ [9,10]. The patch-pipette solution contained: 110 mM caesium sulphate; 0.5 mM $CaCl_2$; 2 mM $MgCl_2$; 5 mM EGTA; 5 mM HEPES; 5 mM tetraethylammonium; 5 mM ATP (magnesium salt); and 1 mM guanosine 5′-*O*-(2-thiodiphosphate) (GDP-β-S), unless otherwise mentioned. In some experiments, a patch-pipette solution containing: 135 mM K-gluconate; 5 mM KCl; 0.5 mM $CaCl_2$; 2 mM $MgCl_2$; 5 mM EGTA; 5 mM HEPES; and 5 mM Mg-ATP, was used. The holding potential (V_H) used was −70 mV. Signals were amplified with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Data were low-pass filtered at 5 kHz, digitized at 333 kHz with an A/D converter, stored and analyzed with a personal computer using pCLAMP 6 and Axo-Graph (Ver. 4.0) data acquisition program (Axon Instruments). mEPSCs were analyzed, as done previously [10]. Stimuli (duration, 100 μs) to elicit EPSCs were given to the dorsal root at a frequency of 0.2 Hz (unless otherwise stated) via a suction electrode; the intensities used were 1.2–1.5 times the threshold required to elicit an EPSC in the Aδ-afferent or C-afferent fibres.

Drugs were applied by superfusion with a change in solutions in the recording chamber, being complete within 20 s. Drugs used were GDP-β-S (Sigma, St Louis, MO), anandamide, R(+)-WIN-2 mesylate (Research Biochemicals International, Natick, MA), and capsaicin (Wako, Japan). Anandamide, WIN-2 and capsaicin were first resolved in an organic solvent (ethanol and dimethyl sulphoxide, respectively, were used for the first and latter two) at concentrations of 10, 5 and 1 mM, respectively, and then diluted to the desired concentration in Krebs solution immediately before use. All numerical data were expressed as the means ± SE. Statistical significance was determined as $P < 0.05$ using the Student's paired or unpaired *t*-test. In all cases, *n* refers to the number of neurones tested.

Whole-cell recordings were obtained from 47 SG neurones; stable recordings could be obtained from slices maintained in vitro for more than 12 h, and recordings could be made from single SG neurones for up to 4 h. All SG neurones examined exhibited mEPSCs at −70 mV, as reported previously [8,9,10,20]. When examined using the patch-pipette solution containing K-gluconate, anandamide (0.01, 0.1, 1 and 10 μM; $n = 3$, 3, 1 and 4, respectively) or WIN-2 (5 and 10 μM; $n = 2$ in each of them) did not change holding currents in SG neurones.

In 79% of 41 neurones examined, stimulating the dorsal root with a strength of more than 20 μA (sufficient to activate Aδ-fibres) elicited glutamatergic monosynaptic EPSCs which displayed no failure and no change in latency when stimulated at 20 Hz, as reported previously [10]. The conduction velocity (CV) estimated from the EPSC latency averaged 4.4 ± 0.2 m/s (range, 2.2–7.1 m/s; $n = 33$); this was within the Aδ-fibre range obtained from experiments in rat dorsal root ganglion (DRG) neurones [2,13]. The monosynaptic Aδ-afferent eEPSCs had a mean amplitude of 200 ± 20 pA (67–508 pA). In 21 (64%) out of the 33 neurones examined, superfusing anandamide (10 μM) inhibited the peak amplitude of the Aδ-eEPSC in a reversible manner (Fig. 1) with an extent of >5%; the inhibition averaged 32 ± 4% in magnitude. The remaining neurones exhibited a change less than this in the amplitude. On the other hand, in 75% of 18 neurones tested, stimuli with a strength larger than 160 μA (enough to recruit C-fibres) induced glutamatergic monosynaptic eEPSCs which had no failure when stimulated at 2 Hz, as reported previously [10]. When estimated from the eEPSC latency, the CV averaged 0.49 ± 0.03 m/s (0.3–0.6 m/s; $n = 13$), values comparable with those of C-fibres [2,13]. The monosynaptic C-afferent eEPSCs had a mean amplitude of 172 ± 28 pA (59–394 pA). In nine (69%) out of the 13 neurones examined, the C-eEPSC was not affected (<5% in amplitude) by anandamide (10 μM), as seen in Fig. 2A. Although the remaining neurones exhibited an inhibition of >5%, this magnitude (17 ± 3%) was less than that of Aδ-eEPSC inhibition ($P < 0.05$; see Table 1). Similar actions on Aδ- and C-eEPSCs were observed by superfusing WIN-2 (5 μM). Aδ-eEPSCs were inhibited by WIN-2 with an extent of 51 ± 18% in four out of nine neurones examined, while C-eEPSCs were depressed in three of five neurones by 10 ± 1%, a value less than that of Aδ-eEPSC ($P < 0.05$). The remaining neurones exhibited changes of less than 5%

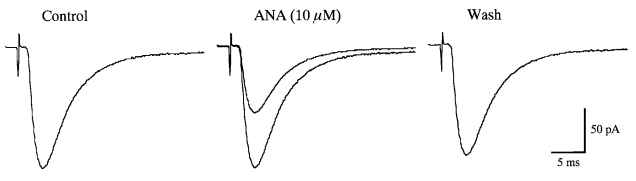

Fig. 1. Action of anandamide (ANA; 10 μM) on monosynaptic eEPSCs in a SG neurone. Average traces of six consecutive Aδ-eEPSCs are shown (measured for 30 s; stimulated at 0.2 Hz) before (left), during the action of ANA (middle; where control Aδ-eEPSC in the left is superimposed for comparison), and 5 min after washout of ANA (right). The intensity of the stimuli used was 40 μA; CV = 5.1 m/s. $V_H = -70$ mV.

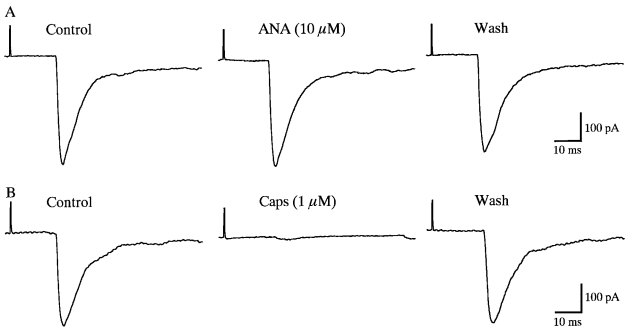

Fig. 2. Actions of anandamide (ANA; 10 μM) and capsaicin (Caps; 1 μM) on monosynaptic C-fibre eEPSCs in a SG neurone. (A) Average traces of six consecutive C-eEPSCs (measured for 30 s; stimulated at 0.2 Hz) before (left), during the action of ANA (middle), and 5 min after washout of ANA (right). (B) Average traces of six consecutive C-eEPSCs (measured for 30 s; stimulated at 0.2 Hz) before (left), during the action of Caps (middle), and 10 min after washout of Caps (right). (A) and (B) were obtained from the same neurone; the intensity of the stimuli used and CV were 480 μA and 0.5 m/s, respectively. $V_H = -70$ mV.

in Aδ- or C-eEPSC amplitudes. Different from such an inhibitory action on eEPSCs, anandamide (10 μM) did not affect the amplitude and frequency of mEPSCs ($n = 6$); they were, respectively, 8.3 ± 1.9 pA and 5.7 ± 1.5 Hz in the control, and 8.1 ± 2.0 pA and 5.8 ± 1.7 Hz at 2 min following its application; each was obtained from mEPSCs measured for 1 min.

As reported previously by Yang et al. [21], capsaicin (1 μM) largely depressed the amplitude of C-eEPSC while almost not changing the Aδ-eEPSC amplitude (90 and 91% of control; not shown). In the neurones where C-eEPSCs were depressed, anandamide (10 μM) was without the action, as seen in Fig. 2; the peak amplitudes in the presence of capsaicin and anandamide were 49 ± 18 and 95 ± 6% ($n = 3$) of control, respectively. Table 1 summarizes the results of the actions of capsaicin on Aδ- and C-eEPSC amplitudes, together with those of anandamide.

The present study demonstrated that anandamide inhibits monosynaptic EPSCs evoked in SG neurones in adult rats, as reported in juvenile rats by Morisset and Urban [12]. This anandamide action appears to be due to the activation of the cannabinoid receptor, because WIN-2 exhibited a similar effect. SG neurones express inwardly-rectifying K^+ channels activated by nociceptin [9]. Although Mackie et al. [11] have reported a cannabinoid-activated, inwardly-rectifying K^+ channel, anandamide and WIN-2 did not change holding currents in adult rat SG neurones, indicating the absence of such a channel in postsynaptic neurones, as seen in rat PAG [18] and SG neurones (juvenile) [12]. There were some differences in the cannabinoid action between adult and juvenile rats. The cannabinoid action in adult rats was not observed in all neurones tested, different from that in juvenile rats where WIN-2 was effective in all neurones examined ($n = 8$). Furthermore, there was a distinction in the action of cannabinoids on mEPSC frequency. Although anandamide inhibited mEPSC frequency in juvenile rats, this was not seen in adult rats. These differences may be due to a developmental change in the expression of the cannabinoid receptor in primary-afferent fibre and also interneurone axon terminals innervating SG neurones.

The current study revealed for the first time that Aδ-fibre eEPSCs are more sensitive to anandamide than C-fibre ones in terms of the population of cells exhibiting its effectiveness (>5%) and of the average magnitude of its depression (see Table 1). Since anandamide did not change the mEPSC amplitude, its action on eEPSCs is presynaptic in origin and thus possibly due to the activation of the cannabinoid receptor existing in primary-afferent terminals. Activation of this receptor would inhibit presynaptic voltage-gated Ca^{2+} channels, leading to a decrease in the release of L-glutamate from nerve terminals, because Ross et al. [15] have reported an inhibition of Ca^{2+} currents by anandamide or WIN-2 in rat DRG neurones. More effectiveness of anandamide on Aδ-fibre than C-fibre EPSCs may be consistent with the observations that CB1 receptor messenger RNA is more expressed in large than small DRG neurones [6], and also that neonatal capsaicin treatment known to destroy C-fibres decreased by only 16% the number of cannabinoid binding sites [5].

There is much evidence supporting an idea that anandamide may be an endogenous ligand for the capsaicin receptor (see [17] for review). In support of this idea in the spinal dorsal horn, Smart et al. [16] have demonstrated that anandamide activates capsaicin receptors expressed on

Table 1
Proportions of SG neurones where anandamide[a] or capsaicin[b] inhibited the amplitude of monosynaptic Aδ-fibre and C-fibre eEPSCs by >5%, and averaged magnitudes of the inhibitions

	Aδ-eEPSC		C-eEPSC	
	Proportion[c]	Inhibition (%)	Proportion[c]	Inhibition (%)
Anandamide	21/33 (64)	32 ± 4 ($n = 21$)	4/13 (31)	17 ± 3 ($n = 4$)
Capsaicin	0/2 (0)	0	3/3 (100)	51 ± 18 ($n = 3$)

[a] Anandamide, 10 μM.
[b] Capsaicin, 1 μM.
[c] Values in parentheses represent percentages.

neonatal rat DRG neurones in culture. If so, anandamide will exhibit a similar action on glutamatergic transmission to that of capsaicin. However, this was not the case, because capsaicin inhibited C-fibre but not Aδ-fibre eEPSCs (see Table 1 and also [21]). Furthermore, the mEPSC frequency was not changed by anandamide whereas this was markedly potentiated by capsaicin [20]. These observations suggest that anandamide may not be an endogenous agonist for the capsaicin receptor existing in primary-afferent terminals in the adult rat SG. Ahluwalia et al. [1] have demonstrated a colocalization of the CB1 cannabinoid and capsaicin receptor in many cultured rat DRG neurones. The possibility cannot be ruled out that anandamide did not reach the capsaicin receptor at concentrations high enough to activate this receptor because of an enzymatic hydrolysis of anandamide or its removal mechanism from the extracellular space by a transporter (see [3] for review).

The finding revealed in this work would contribute to at least a part of the antinociception induced by intrathecally-administered cannabinoids. Different from baclofen or nociceptin, cannabinoids may inhibit more effectively fast than slow conducting pain sensations.

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